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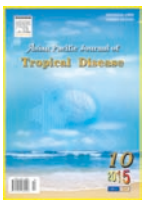

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Interferon-γ +874A/T polymorphism associated with *Toxoplasma gondii* seropositivity in HIV patients

Sri Haryati[1,2,3], Afiono Agung Prasetyo[1,2,4*], Ratna Sariyatun[1,2], Yulia Sari[1,2,3], Murkati[1,2,3]

[1]*A-Infection, Genomic, Immunology and Cancer Research Group, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta, Indonesia*

[2]*Center of Biotechnology and Biodiversity Research and Development, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta, Indonesia*

[3]*Department of Parasitology, Faculty of Medicine, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta, Indonesia*

[4]*Department of Microbiology, Faculty of Medicine, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta, Indonesia*

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ABSTRACT

Objective: To investigate the association of polymorphisms in genes that code for interferon-γ (IFN-γ) and interleukin-10 (IL-10), which play important roles in *Toxoplasma gondii* (*T. gondii*) infection, with the occurrence of *T. gondii* co-infection in HIV patients.
Methods: The IFN-γ +874A/T and IL-10 -1082A/G polymorphism statuses of 306 HIV seropositive samples were characterized using PCR. The polymorphism statuses were analyzed together with the clinical data for each patient.
Results: Immunoglobulin M anti-*T. gondii* seropositivity was associated with high IL-10 levels [adjusted odds ratio (OR): 0.4, 95% confidence intervals (*CI*): 0.181–0.825; $P = 0.014$], but not with either the IL-10 -1082A/G or IFN-γ +874A/T polymorphism. In addition, the IFN-γ +874A allele was associated with immunoglobulin G (IgG) anti-*T. gondii* seropositivity (OR: 1.5, 95% *CI*: 1.043–2.193; $P = 0.029$). In patients with $CD4^+$ T cell levels ⩾ 200 cells/μL, the IFN-γ +874 AA genotype was associated with IgG anti-*T. gondii* seropositivity (adjusted OR: 2.5, 95% *CI*: 1.278–4.950; $P = 0.008$).
Conclusions: The IFN-γ +874A/T polymorphism is associated with IgG anti-*T. gondii* seropositivity. This polymorphism might be useful to predict the susceptibility of HIV patients to toxoplasmosis.

1. Introduction

Toxoplasma gondii (*T. gondii*) is the most prevalent and successful intracellular protozoan parasite worldwide[1,2]. This parasite is globally distributed, appearing as a long-lasting persistent infection in 30%–80% of the global human population and it is more prevalent in tropical areas, such as Indonesia[3,4]. *T. gondii* infection is asymptomatic in healthy people. However, this infection is life-threatening in HIV patients, in whom a latent infection with this parasite is commonly reactivated due to the immunodeficiency[4,5]. Notably, *T. gondii* has emerged as one of the most common opportunistic infections associated with AIDS during the past decade, reflecting the prevalence of latent infection in HIV-infected patients, which ranges from 3% to 97%[1,6]. In most cases, toxoplasma encephalitis is the most common manifestation of this disease and represents a leading cause of death among HIV patients. Toxoplasma encephalitis affected 50% of patients in Europe and Africa during the pre-highly active anti-retroviral therapy era[7]. The advent of highly active anti-retroviral therapy has decreased the occurrence of *T. gondii*-related manifestations in HIV patients[7], though reactivation remains a clinical challenge once a patient develops resistance.

Interferon-γ (IFN-γ) and interleukin-10 (IL-10) are two vital immune system cytokines secreted in response to *T. gondii* infection[2,3,8]. IFN-γ is the principal cytokine for host defense against *T. gondii*[8]. In contrast, IL-10 plays a key role in the regulation of inflammatory responses and cell-mediated immunity against this parasite, such as the inhibition of the production of cytokines required for the optimal production of IFN-γ, including tumor necrosis factor-α, IL-1, and IL-12[2,3,9-12]. The balance of the pro- and anti-inflammatory responses (IFN-γ and IL-10, respectively) is important for host survival[3,13]; IL-10 deficiency impairs immune homeostasis in response to infection-induced immunopathology, whereas high IL-10 production represses the immunity against the

*Corresponding author: Afiono Agung Prasetyo, MD, PhD, Department of Microbiology, Faculty of Medicine, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta 57126, Indonesia.
Tel/Fax: +62271 632489
E-mails: afie.agp.la@gmail.com, afie@staff.uns.ac.id, afieagp@yahoo.com

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parasite, including IFN-γ-mediated responses, thereby enhancing the susceptibility to the progression of *T. gondii* infection[9].

Host genetic variations influence both IFN-γ and IL-10 production and are involved in various inflammatory and infectious diseases, such as *T. gondii* infection[14]. A single nucleotide polymorphism has been identified in the first intron of the IFN-γ gene at position +874A/T (rs2430561) located on chromosome 12q24.1. This mutation modulates the binding of nuclear factor-κB, thereby causing high (TT), intermediate (TA), or low (AA) IFN-γ production[15-18]. In addition, single nucleotide polymorphism-1082A/G (rsl800896) in the IL-10 gene promoter might influence IL-10 transcription and secretion[19,20]. The -1082G allele is associated with a higher IL-10 serum concentration[21], whereas the -1082A allele is associated with lower IL-10 production[22]. Although both IFN-γ and IL-10 are crucial in *T. gondii* infection, the influence of IFN-γ and IL-10 polymorphisms on *T. gondii* infection is poorly understood. Here, we examined the influence of IFN-γ +874A/T and IL-10 -1082A/G polymorphisms on the susceptibility of HIV-infected patients to *T. gondii* infection.

2. Materials and methods

2.1. Study population

Since 2009, our research group has performed a molecular epidemiology study of human blood-borne viruses based on the collection of epidemiological data and blood samples from high-risk communities, such as drug-abusing inmates in prisons and jails, men having sex with men in Surakarta and the surrounding cities, commercial sex workers, injection drug users and people who have heterosexual contact with HIV-positive partners in Central Java, Indonesia[23,24]. During 2010-2012, 306 HIV-positive samples were collected. Blood samples were obtained from all patients and subjected to routine hematological and $CD4^+$ T cell testing, the detection of immunoglobulin M (IgM) and immunoglobulin G (IgG) anti-*T. gondii* antibodies, and IFN-γ and IL-10 levels, as described previously[23-27]. Approval was obtained from the institutional ethical committee review boards of the Faculty of Medicine of Sebelas Maret University and Dr. Moewardi (General Hospital, Central Java Province, Indonesia). Written informed consent was obtained from each individual participating in the study. All procedures were conducted according to the principles of the Declaration of Helsinki.

2.2. Detection of IFN-γ +874A/T and IL-10 -1082A/G polymorphisms

Genomic DNA from each patient was isolated from peripheral blood using a high pure PCR template preparation kit (Roche Diagnostics, Mannheim, Germany). The IFN-γ +874A/T and IL-10 -1082A/G polymorphisms were genotyped via PCR using the FastStart HiFi PCR System dNTPack (Roche) with primer pairs previously described for IFN-γ +874A/T and IL-10 -1082A/G polymorphisms[28,29]. To avoid false-negative results, an internal control primer amplifying a 429-bp region of the human growth hormone gene was used for all PCR reactions[26]. The PCR products were subjected to electrophoresis in ethidium bromide-stained 3% agarose gels for 30 min at 100 V. The IFN-γ +874A/T and IL-10 -1082A/G allele frequencies were estimated using the direct counting method.

2.3. Statistical analysis

The *Chi*-square test was used to assess the Hardy-Weinberg equilibrium (HWE) of the IFN-γ +874A/T and IL-10 -1082A/G genotype distributions in the study population. The *Chi*-square test, Fisher's exact test and Kolmogorov-Smirnov test were used to analyze categorical variables. Logistic regression was used to reveal associations between polymorphism status and patient susceptibility to *T. gondii* infection. All data were analyzed using a 95% confidence interval (*CI*) and a two-tailed *P* value of < 0.05 was considered to indicate significant differences. All statistical analyses were performed using SPSS software version 21 (IBM Corp, Armonk, NY, USA).

3. Results

3.1. Study population

HIV-positive samples were collected from 174 (56.9%) males and 132 (43.1%) females; the patients were aged 19–72 years (Table 1). Some patients (31.4%; 96/306) were severely immunodeficient, with $CD4^+$ T cell counts < 200 cells/μL[30]. Among the subjects, patients aged ⩽ 30 years tended to have a high $CD4^+$ T cell percentage (> 28%) [odds ratio (OR) 2.0, 95% *CI* 1.096–3.536; $P = 0.022$] and a $CD4^+$ T cell count > 500 cells/μL (OR 2.1, 95% *CI* 1.252–3.630; $P = 0.005$).

Table 1
Baseline data of the study population among 306 HIV-positive samples.

Characteristics		*n* (%)
Gender	Male	174 (56.9)
	Female	132 (43.1)
Mean age ± SD in years		35.9 ± 8.9
Age category in years	⩽ 30	78 (25.5)
	31–40	155 (50.6)
	41–50	51 (16.7)
	> 50	22 (7.2)
Risk status	High-risk couples	104 (34.0)
	IDUs	54 (17.7)
	CSWs	35 (11.4)
	Customers of CSWs	89 (29.1)
	MSM	24 (7.8)
Anemia*		160 (52.3)
Erythrocyte ($\times 10^6$/μL)	< 4.4M/< 3.9F	241 (78.8)
	4.4–5.7M/3.9–5.0F	61 (19.9)
	> 5.7M/> 5.0F	4 (1.3)
Leukocyte ($\times 10^6$/μL)	< 3.9M/< 3.7F	52 (17.0)
	3.9–11.1M/3.7–9.5F	242 (79.1)
	> 11.1M/> 9.5F	12 (3.9)
Thrombocyte ($\times 10^5$/μL)	< 1.5	7 (2.3)
	1.5–4.0	253 (82.7)
	> 4.0	46 (15.0)
Hematocrit (%)	< 33	79 (25.8)
	33–45	222 (72.6)
	> 45	5 (1.6)
$CD4^+$ T cell (%)	< 14	118 (38.5)
	14–28	122 (39.9)
	> 28	66 (21.6)
$CD4^+$ T cell (cells/μL)	< 200	96 (31.4)
	200–500	112 (36.6)
	> 500	98 (32.0)
IFN-γ (pg/mL)	⩽ 10	289 (94.4)
	>10	17 (5.6)
IL-10 (pg/mL)	⩽ 10	207 (67.6)
	>10	99 (32.4)

*: Patients were considered anemic when the hemoglobin levels were < 13.3 g/dL (M) and < 11.8 g/dL (F). M: Male; F: Female; IDUs: Injection drug users; CSWs: Commercial sex workers; MSM: Men have sex with men.

3.2. IgM/IgG anti-T. gondii seropositivity

In total, 22.5% (69/306) and 31.7% (97/306) of patients were IgM and IgG anti-*T. gondii* seropositivity, respectively. The proportion of IgM and IgG anti-*T. gondii* positivity differed by age ($P < 0.001$ and $P = 0.002$, respectively); IgM anti-*T. gondii* positivity was less frequent in patients aged ⩽ 30 years, whereas the IgG anti-*T. gondii* positivity was more common in older patients (Tables 2 and 3). In addition, IgM anti-*T. gondii*, but not IgG, was associated with $CD4^+$ T cell levels (Table 3). However, the rate of IgM anti-*T. gondii* seropositivity was differentially associated with $CD4^+$ T cell count and percentages were discordant. While IgM anti-*T. gondii* positivity tended to be negatively associated with $CD4^+$ T cell percentage, it was less common in patients with $CD4^+$ T cell < 100 cells/μL (Tables 2 and 3). Furthermore, the rates of IgM and IgG anti-*T. gondii* seropositivity did not correlate with the plasma IFN-γ levels ($P = 0.771$ and $P = 0.069$, respectively), whereas a significant association between IgM anti-*T. gondii* seropositivity and the IL-10 levels was observed (Table 3).

Table 2

Distribution of IgM ($N = 69$) and IgG ($N = 97$) anti-*T. gondii* seropositivity with respect to the clinical status of the HIV patients. *n* (%).

Characteristics		*n*	IgM anti-*T. gondii* (+)	IgG anti-*T. gondii* (+)
Gender	Male	174	37 (53.6)	64 (66.0)
	Female	132	32 (46.4)	33 (34.0)
Age in years	⩽ 30	78	4 (5.8)	13 (13.4)
	31–40	155	43 (62.3)	59 (60.8)
	41–50	51	16 (23.2)	14 (14.5)
	> 50	22	6 (8.7)	11 (11.3)
$CD4^+$ T cell percentage	< 14	118	36 (52.2)	37 (38.1)
	14–28	122	30 (43.5)	35 (36.1)
	> 28	66	3 (4.3)	25 (25.8)
$CD4^+$ T cell count (cells/μL)	< 200	96	19 (27.6)	28 (28.9)
	200–500	112	33 (47.8)	36 (37.1)
	> 500	98	17 (24.6)	33 (34.0)
$CD4^+$ T cell < 100 cells/μL		40	3 (4.3)	12 (12.4)
IFN-γ (pg/mL)	⩽ 10	289	66 (95.7)	95 (97.9)
	> 10	17	3 (4.3)	2 (2.1)
IL-10 (pg/mL)	⩽ 10	207	52 (75.4)	69 (71.1)
	> 10	99	17 (24.6)	28 (28.9)

3.3. IFN-γ +874A/T and IL-10 -1082A/G polymorphisms

In total, 49.0% (150/306), 34.0% (104/306) and 17.0% (52/306) of patients harbored IFN-γ +874 AA, AT, and TT genotypes, respectively, resulting in A and T allele frequencies of 0.660 and 0.340, respectively. In addition, IL-10 -1082 AA, AG and GG genotypes were observed in 28.4% (87/306), 58.2% (178/306) and 13.4% (41/306) of patients, respectively, with A and G allele frequencies of 0.575 and 0.425, respectively. The distribution of IFN-γ +874A/T ($P < 0.001$) and IL-10 -1082A/G ($P = 0.001$) genotype polymorphisms in HIV-positive patients deviated from the expected frequencies for HWE. Interestingly, the IFN-γ +874T allele most commonly appeared with the IL-10 -1082A allele (OR = 1.7, 95% *CI* 1.327–2.168; $P < 0.001$) and less commonly appeared with the IL-10 -1082G allele (OR = 0.6, 95% *CI* 0.461–0.753; $P < 0.001$). Similarly, individuals bearing the IFN-γ +874T allele (AT/TT) frequently carried the IL-10 -1082A allele (AA/AG) (OR = 2.5, 95% *CI* 1.253–5.088; $P = 0.008$), whereas patients with the IFN-γ +874A allele (AA/AT) commonly harbored the IL-10 -1082G allele (AG/GG) (OR = 4.7, 95% *CI* 2.525–8.797; $P < 0.001$).

3.4. Association of IFN-γ +874A/T and IL-10 -1082A/G polymorphisms with cytokine levels, $CD4^+$ T cell counts and IgM/IgG anti-T. gondii seropositivity

Neither the IFN-γ+874A/T nor the IL-10-1082A/G genotype distribution were correlated with the IFN-γ ($P = 0.182$ and $P = 0.560$, respectively) or IL-10 levels ($P = 0.621$ and $P = 0.519$, respectively). In contrast, the proportion of IFN-γ+874A/T and IL-10-1082A/G genotypes was significantly correlated with the $CD4^+$ T cell percentage ($P = 0.001$ and $P = 0.037$, respectively) and $CD4^+$ T cell count ($P = 0.003$ and $P < 0.001$, respectively). The IFN-γ+874A allele was associated with a low $CD4^+$ T cell count (< 200 cells/μL) (OR 1.5, 95% *CI* 1.020–2.146; $P = 0.039$) and percentage (< 14%) (OR 1.8, 95% *CI* 1.247–2.544; $P = 0.002$). A low $CD4^+$ T cell count was also associated with the IL-10 -1082A allele (OR 1.9, 95% *CI* 1.307–2.675; $P < 0.001$).

The IFN-γ +874A/T and IL-10 -1082A/G allele frequencies did not correlate with IgM anti-*T. gondii* seropositivity (Table 4) in either males ($P = 0.800$ and $P = 0.216$, respectively) or females ($P = 0.266$ and $P = 0.155$, respectively), whereas the IFN-γ +874A allele was associated with a higher likelihood to harbor IgG anti-*T. gondii* (Table 4). Independent analyses of the male and female groups showed that the rate of IgG anti-*T. gondii* seropositivity was only correlated with the IFN-γ +874A allele frequencies in males ($P = 0.014$), but not in females ($P = 0.965$). A multivariate analysis of the male group considering the age, risk status and clinical condition of the patient showed that the IFN-γ +874A/T genotype did not significantly contribute to IgG anti-*T. gondii* seropositivity ($P = 0.086$).

To overcome pitfalls due to potentially false IgM/IgG anti-*T. gondii* seronegativity in immunosuppressed patients, a analysis of a group of patients with $CD4^+$ T cell counts ⩾ 200 cells/μL ($n = 210$)

Table 3

Factors associated with IgM and IgG anti-*T. gondii* seropositivity in HIV patients.

Characteristics		IgM anti-*T. gondii* (+)				IgG anti-*T. gondii* (+)			
		OR (95% *CI*)	*P*	aOR (95% *CI*)	*P*	OR (95% *CI*)	*P*	aOR (95% *CI*)	*P*
Age in years	⩽ 30	0.1 (0.048–0.386)	< 0.001	0.1 (0.044–0.446)	0.001	0.3 (0.178–0.659)	0.001	-	-
	31–40	1.8 (1.065–3.198)	0.028	-	-	1.8 (1.119–2.984)	0.015	2.3 (1.334–4.070)	0.003
	> 50	-	-	-	-	2.3 (0.961–5.513)	0.060	2.8 (1.042–7.729)	0.041
Gender/male		-	-	-	-	1.7 (1.059–2.878)	0.028	1.8 (1.048–3.103)	0.033
$CD4^+$ T cell	< 100 cells/μL	0.2 (0.073–0.823)	0.015	0.1 (0.034–0.514)	0.003	-	-	-	-
	< 14%	2.1 (1.198–3.549)	0.008	5.2 (2.120–12.830)	< 0.001	-	-	-	-
	> 28%	0.1 (0.038–0.414)	< 0.001	0.1 (0.021–0.374)	0.001	-	-	-	-
	IL-10 > 10 pg/mL	0.6 (0.336–1.137)	0.120	0.4 (0.181–0.825)	0.014	-	-	-	-

aOR: Adjusted odds ratio.

Table 4

Frequencies of IFN-γ +874A/T IL-10 -1082A/G genotypes and alleles according to the IgM and IgG anti-*T. gondii* seropositivity of the HIV patients.

Polymorphism		IgM anti-*T. gondii* (+) (*n* = 69)			IgG anti-*T. gondii* (+) (*n* = 97)		
		Frequencies	OR (95% *CI*)	*P*	Frequencies	OR (95% *CI*)	*P*
IFN-γ +874 genotypes	TT (*n* = 52)	23.2 (16/69)	1.0	0.260[a]	12.4 (12/97)	1.0	0.143[a]
	AT (*n* = 104)	29.0 (20/69)	0.5 (0.249–1.151)		30.9 (30/97)	1.4 (0.624–2.925)	
	AA (*n* = 150)	47.8 (33/69)	0.6 (0.314–1.284)		56.7 (55/97)	1.9 (0.934–3.987)	
	AA (*n* = 150)	47.8 (33/69)	1.0	0.822[b]	56.7 (55/97)	1.0	0.067[b]
	AT + TT (*n* = 156)	52.2 (36/69)	1.1 (0.622–1.819)		43.3 (42/97)	0.6 (0.392–1.034)	
	TT (*n* = 52)	23.2 (16/69)	1.0	0.120[b]	12.4 (12/97)	1.0	0.142[b]
	AA + AT (*n* = 254)	76.8 (53/69)	0.6 (0.306–1.150)		87.6 (85/97)	1.7 (0.836–3.362)	
IFN-γ +874 alleles	A (*n* = 404)	62.3 (86/138)	0.8 (0.547–1.203)	0.298[b]	72.2 (140/194)	1.5 (1.043–2.193)	0.029[b]
	T (*n* = 208)	37.7 (52/138)	1.2 (0.831–1.828)		27.8 (54/194)	0.7 (0.456–0.959)	
IL-10 -1082 genotypes	GG (*n* = 41)	23.2 (16/69)	1.0	<0.001[a]	9.3 (9/97)	1.0	0.137[a]
	AG (*n* = 178)	24.6 (17/69)	0.2 (0.074–0.368)		66.0 (64/97)	2.0 (0.897–4.444)	
	AA (*n* = 87)	52.2 (36/69)	1.1 (0.516–2.356)		24.7 (24/97)	1.4 (0.564–3.254)	
	AA (*n* = 87)	52.2 (36/69)	1.0	<0.001[b]	24.7 (24/97)	1.0	0.330[b]
	AG + GG (*n* = 219)	47.8 (33/69)	0.3 (0.143–0.442)		75.3 (73/97)	1.3 (0.759–2.270)	
	GG (*n* = 41)	23.2 (16/69)	1.0	0.007[b]	9.3 (9/97)	1.0	0.149[b]
	AA + AG (*n* = 265)	76.8 (53/69)	0.4 (0.195–0.783)		90.7 (88/97)	1.8 (0.808–3.866)	
IL-10 -1082 alleles	A (*n* = 352)	64.5 (89/138)	1.5 (0.984–2.158)	0.060[b]	57.7 (112/194)	1.0 (0.718–1.430)	0.941[b]
	G (*n* = 260)	35.5 (49/138)	0.7 (0.463–1.016)		42.3 (82/194)	1.0 (0.699–1.393)	

[a]: Frequencies of genotypes among patients with $CD4^+$ T cell counts < 14% and those with higher levels were compared using the *Chi*-square test for 3 × 2 contingency tables; [b]: Frequencies of genotypes between cases and controls were compared using the *Chi*-square test for 2 × 2 contingency tables to analyze AA and TT genotypes with respect to AT + TT and AT + AA, respectively.

was conducted. Similar to the analysis of the overall group, the IFN-γ +874A allele remained associated with a higher rate of IgG anti-*T. gondii* seropositivity (OR 1.8, 95% *CI* 1.181–2.862; *P* = 0.007). After considering the age, risk status, $CD4^+$ T cell count, IFN-γ and IL-10 levels and other hematological profiles of the patient as potential confounders, the IFN-γ +874 AA genotype was associated with a higher proportion of IgG anti-*T. gondii* seropositivity (aOR 2.5, 95% *CI* 1.278–4.950; *P* = 0.008). In addition, IgM anti-*T. gondii* seropositivity did not correlate with either IFN-γ +874A/T or IL-10 -1082A/G polymorphism.

4. Discussion

Due to the high risk of the fatal reactivation of past *T. gondii* infection in immunocompromised individuals, the detection and monitoring of anti-*T. gondii* antibodies has been recommended for newly diagnosed HIV-positive patients and prophylaxis should be considered in patients with $CD4^+$ T cell counts < 200 cells/μL[6]. However, diagnosis is difficult because clinical manifestations are non-specific and anti-*T. gondii* antibody concentrations are low. Furthermore, diagnosis is even more challenging in low-resource settings where radiography examination is inadequate[31,32]. Indeed, reactivation is associated with high morbidity and mortality[5]. This issue becomes particularly important in countries where HIV and *T. gondii* are prevalent, such as Indonesia[33,34]. Therefore, identifying the factors associated with the occurrence of *T. gondii* reactivation and reinfection in HIV patients will be useful to prevent the incidence of this disease.

The level of $CD4^+$ T cells, which are essential regulators of the immune reactions during toxoplasmosis[3,8,35], has been suggested as an indicator for the susceptibility to reactivation[36]. The risk for latent *T. gondii* infection reactivation increases for $CD4^+$ T cell counts below 100 cells/μL and the incidence of *T. gondii* seropositivity is also higher in HIV-infected individuals with $CD4^+$ T cell counts of 200–499 cells/μL than in individuals with counts > 500 cells/μL[36,37]. Similarly, the results of this study showed that IgM anti-*T. gondii* seropositivity is more common in patients with 200–500 $CD4^+$ T cells/μL than in patients with > 500 $CD4^+$ T cells/μL; furthermore, the frequency of IgM anti-*T. gondii* seropositivity gradually decreases with the $CD4^+$ T cell percentage increases. However, IgG anti-*T. gondii* seropositivity did not correlate with the $CD4^+$ T cell levels[38-40]. Interestingly, we observed that the rate of IgM anti-*T. gondii* was significantly lower in patients with $CD4^+$ T cell counts < 100 cells/μL than in patients with higher $CD4^+$ T cell counts. This finding likely indicates false IgM anti-*T. gondii* seronegativity resulting from the severe immunodeficiency of patients with $CD4^+$ T cell counts < 100 cells/μL, which impairs the production of IgM anti-*T. gondii*[5,40]. Unfortunately, we could not confirm the presence of *T. gondii* infection in these patients. Further studies should analyze the appropriateness of IgM anti-*T. gondii* testing in patients with severe immunosuppression because these tests might underestimate the diagnosis of *T. gondii* co-infection in HIV patients.

In our study, the rate of IgM anti-*T. gondii* seropositivity was lower than that of IgG anti-*T. gondii* seropositivity, which is consistent with previous reports[40,41]. The proportions of both antibodies were the smallest in patients aged ⩽ 30 years and increased in older patients. These results are consistent with previous studies showing that the prevalence of the *T. gondii* serological marker increases with age[42-45]. These results reflect higher $CD4^+$ T cells counts in young subjects and lower $CD4^+$ T cell counts in older patients[25,43]. Moreover, older individuals have potentially been exposed to the parasite for a longer time, explaining the higher serological prevalence observed in this group[46].

The IL-10 -1082A/G alleles and IgM/IgG anti-*T. gondii* seropositivity were not correlated in the overall population, whereas the IFN-γ +874A allele was associated with a higher likelihood of harboring IgG anti-*T. gondii*. To examine potential pitfalls in analyzing IgM and IgG anti-*T. gondii* due to the aforementioned false seronegativity in immunosuppressed patients, we analyzed the association between both polymorphisms and serological markers of *T. gondii* in patients with $CD4^+$ T cell counts ⩾ 200 cells/μL. In this group, patients carrying the IFN-γ +874 AA genotype were

more susceptible to IgG anti-*T. gondii* seropositivity than patients carrying other genotypes. Thus, the IFN-γ+874A/T polymorphism status might be useful to predict the likelihood of IgG anti-*T. gondii* positivity in HIV patients. In addition, the results of the present study showed no association between the IFN-γ +874A/T and IL-10 -1082A/G polymorphisms and IFN-γ and IL-10 levels, respectively. This lack of association might reflect the fact that the HIV infection influences the expression of IFN-γ and IL-10[47,48], thereby disguising the effect of either the IFN-γ +874A/T or IL-10 -1082A/G polymorphism on the cytokine levels.

To the best of our knowledge, this is the first study to report the rate of the IFN-γ +874A/T polymorphism in Indonesia. The results of the present study also enriched the data concerning the IL-10 -1082A/G polymorphism in Indonesia and Southeast Asia, which remain limited[27]. The results of the present study might provide further insight into the distribution of polymorphisms in this area, which is beneficial because both polymorphisms have been associated with infectious diseases[15,27,35]. This study also contributed data on IgM and IgG anti-*T. gondii* seropositivity in HIV-infected patients in Indonesia.

The present study has several limitations. First, *T. gondii* infection was defined based on the detection of IgM and IgG anti-*T. gondii*. However, the titers of these antibodies were unknown. As a limitation of serological testing, the false negativity reflecting impaired antibody production in immunodeficient individuals and the window period of the infection could not be excluded. Second, the distribution of both IFN-γ +874A/T and IL-10- 1082A/G genotype polymorphisms did not comply with HWE, reflecting the fact that only HIV patients were enrolled in the present study. Thus, our results do not represent the distribution in the general population. This limitation should be a concern because this specific enrollment potentially affects the associations found in the present study[49]. In addition, risk factors for *T. gondii* infection, such as the clinical stage of HIV infection and behavioral history of each patient (*e.g.*, contact with cats and the consumption of undercooked meats), were not considered whereas these parameters might confound the observed associations. Nevertheless, this study showed that the IFN-γ +874A/T polymorphism might predict the susceptibility of HIV patients for the development of toxoplasmosis. Due to the aforementioned limitations, further research is needed to confirm the findings presented herein.

In conclusion, in HIV patients with $CD4^+$ T cell counts $\geqslant$ 200 cells/μL, the IFN-γ +874 AA genotype is associated with increased risk of IgG anti-*T. gondii* positivity. Characterizing the IFN-γ +874A/T polymorphism status of HIV patients might be useful to predict susceptibility to the development of toxoplasmosis.

Conflict of interest statement

We declare that we have no conflict of interest.

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